

Elevated CO₂, drought and soil nitrogen effects on wheat grain quality

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Summary

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- The likely consequences of future high levels of atmospheric CO₂ concentration on wheat (*Triticum aestivum* L.) grain nutritional and baking quality were determined.
- Two free-air CO₂ enrichment (FACE; 550 mmol mol⁻¹) experiments were conducted at ample (Wet) and limiting (Dry) levels of irrigation, and a further two experiments at ample (High-N) and limiting (Low-N) nitrogen concentrations. Harvested grain samples were subjected to a battery of nutritional and bread-making quality tests.
- The Dry treatment improved grain quality slightly (protein +2%; bread loaf volume +3%). By contrast, Low-N decreased quality drastically (protein -36%; loaf volume -26%). At ample water and N, FACE decreased quality slightly (protein -5%; loaf volume -2%) in the irrigation experiments and there was no change in the nitrogen experiments. At Low-N, FACE tended to make the deleterious effects of Low-N worse (protein -33% and -39%, at ambient CO₂ and FACE, respectively; loaf volume -22% and -29% at ambient CO₂ and FACE, respectively).
- The data suggest that future elevated CO₂ concentrations will exacerbate the deleterious effects of low soil nitrogen on grain quality, but with ample nitrogen fertilizer, the effects will be minor.

Key words: *Triticum aestivum* (wheat) grain, quality, nitrogen, Free air CO₂ enrichment (FACE), drought, water-stress, global change, protein.

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Introduction

The CO₂ concentration of the atmosphere is increasing, and climate modelers have predicted a consequent global warming as well as changes in precipitation patterns. The report of the IPCC (Intergovernmental Panel on Climate Change, 1996) projects CO₂ increasing from present day concentrations of about 370 µmol mol⁻¹ to about 500 µmol mol⁻¹ by the end of this century if emissions are maintained at 1994 levels. They further project that the increase in CO₂ plus that of other radiatively active 'greenhouse' gases – methane, nitrous oxide, chlorofluorocarbons (CFCs), ozone – will cause an increase in global mean temperature of 0.9–3.5°C depending on future emission rates. Some regions might receive increases in precipitation, while others might receive less.

However, such elevated concentrations of CO₂ are also likely to stimulate photosynthesis and the growth of plants, including the yield of agricultural crops (Kimball, 1983, 1986). The stimulation of photosynthesis leads to greater production of carbohydrates, which can accumulate in the leaves (Hendrix, 1992) and possibly other organs. The higher concentration of carbohydrates, relative to that of proteins, suggests that the nutritional value of plants to animals and other organisms at other trophic levels would be decreased. Leaf-eating insects (and other herbivores) are likely to be affected (Akey & Kimball, 1989; Lincoln, 1993; Hesman, 2000), whereas seed-eating animals may not be (Akey *et al.*, 1988) if the quality of other organs is unaffected by elevated CO₂. Therefore, it is very important to ascertain whether or how much the quality of agricultural crops and of other plants – but especially of wheat

(*Triticum aestivum* L.) grain, the world's foremost food and feed crop – will be affected by higher atmospheric CO₂ concentrations in the future.

Of course, to humans, the bread-making quality of future wheat grain is also important, in addition to its nutritional value. Whereas soft wheat flour with lower protein concentration is used to make such foods as cookies, growers usually receive higher prices for hard wheat grain with higher protein concentrations and good ratios of gliadin and glutenin components, traits fundamental to making high-quality leavened breads (Stone & Savin, 1999).

The effects of environmental variables other than CO₂ on wheat grain protein concentration were studied intensively two decades ago. For example, Benzie & Lane (1979, 1981, 1986) assembled data from many experiments in England. For spring wheat, such as studied herein, the protein concentrations ranged from c. 9–16% with the optimum associated with maximum yield from c. 12–15%. About 75% of their values were above the minimum protein concentration for bread making (10% d. wt basis or 11.6% at 14% moisture). The percentages varied with variety, soil type, prior cropping history (especially legume vs nonlegume), and N fertilizer amount, as well as N application timing. However, below the optimum rate, the protein concentration was most sensitive to the N application rate, and they determined that about 56 kg ha⁻¹ of applied N corresponded to an increase in grain protein concentration of 1% (absolute). They also examined weather factors, finding that a 1°C rise in temperature was associated with a 0.4% absolute increase in grain protein concentration, but they did not detect any effect of moisture stress under their English conditions. On the other hand, Barber & Jessop (1987) in Australia and Garrot *et al.* (1994) in Arizona studied irrigated wheat, and they both found that a water stress regime that reduced yields to two-thirds tended to increase grain protein slightly.

Then, within the past decade, several reports have appeared about the effects of elevated CO₂ concentration on wheat grain quality from various chamber-based CO₂ enrichment experiments, as reviewed recently by Lawlor & Mitchell (2000). Many of the experiments reported in the literature found little or no change in wheat grain nutritional or bread-making quality due to elevated levels of CO₂ (Havelka *et al.*, 1984; Williams *et al.*, 1995; Tester *et al.*, 1995; Rogers *et al.*, 1998; Reuveni & Bugbee, 1997; Hakala, 1998 (at higher temperature); Dijkstra *et al.*, 1999). On the other hand, a comparable number of such experiments have found decreases in the protein concentration, which reduced nutritional value and adversely affected baking quality (Conroy *et al.*, 1994 (–1 to –14% over a range of CO₂ and N levels); Thompson & Woodward, 1994 (–28% in monoculture and zero to –20% in competition with weeds); Manderscheid *et al.*, 1995 (–29%); Blumenthal *et al.*, 1996 (–14%); Monje & Bugbee, 1998 (–9%); Hakala, 1998 (–7% at ambient temperature). Therefore, whether or not elevated CO₂ concentration will affect wheat grain quality in the future is an unresolved question.

All of the CO₂ enrichment experiments cited above utilized chambers of various designs to confine the CO₂ around the plants. However, the environment inside chambers is usually not the same as outside, and plants generally grow differently inside (Kimball *et al.*, 1997). Therefore, we can not be confident the chamber-based data reliably represent what the responses of field-grown plants will be. About a decade ago, however, technology for free-air CO₂ enrichment (FACE) was developed (Hendrey, 1993). Although slight changes in the microclimate have been detected at night (Pinter *et al.*, 2000), the FACE technique, which eliminates walls, enables the growing of plants at elevated levels of CO₂ under conditions as representative of future fields as is possible today.

During the 1992–3 and 1993–4 growing seasons, wheat crops were grown using FACE at ample and limiting levels of soil water supply, and similar FACE experiments were conducted during 1995–6 and 1996–7 at ample and limiting levels of soil nitrogen. The wheat grain from these four experiments was sampled and subjected to a battery of tests to determine both nutritional and baking quality. The purpose of this paper is to examine the results from these many quality tests on wheat grain from robust field experiments in order to further elucidate how grain quality is likely to change due to the increasing atmospheric CO₂ concentration.

Materials and Methods

Two experiments were conducted during the 1992–3 and 1993–4 growing seasons to determine the interactive effects of elevated atmospheric CO₂ concentration and limited soil water supply on spring wheat (*Triticum aestivum* L. cv. Yecora Rojo) at the University of Arizona Maricopa Agricultural Center (MAC), Maricopa, Arizona, USA. Two additional experiments were similarly conducted to determine the interactive effects of elevated CO₂ and limited soil nitrogen during the 1995–6 and 1996–7 growing seasons. A field plot plan for the 1992–3 and 1993–4 experiments is presented by Wall & Kimball (1993), and for the 1995–6 and 1996–7 experiments by Kimball *et al.* (1999). Additional details about the methodology are presented by Kimball *et al.* (1995), Hunsaker *et al.* (1996), and Pinter *et al.* (1996, 2000).

CO₂ treatments

Briefly, elevated CO₂ levels were maintained 24 h d⁻¹ all season long in four replicate 25-m-diameter plots using free-air CO₂ enrichment (FACE) technology (Hendrey, 1993). During the first two FACE × H₂O experiments, a constant set-point concentration of 550 μmol mol⁻¹ CO₂ was used, whereas for the later FACE × N experiments, a constant elevation of 200 μmol mol⁻¹ above ambient was imposed. There also were four plots at ambient CO₂ concentration in each experiment. For the two FACE × H₂O experiments these 'Ambient' plots had similar piping to that of the FACE plots,

but there were no blowers installed. As a consequence, these Ambient plots with no forced air movement were 0.6–1.0°C cooler at night than the FACE plots (Pinter *et al.*, 2000). For the later FACE × N experiments, blowers were installed, and microclimatic differences between these ‘Control’ plots and the FACE plots due to differences in apparatus were minimal. The season-long daytime average CO₂ concentrations were 550, 550, 548, and 559 μmol mol⁻¹ CO₂ in the FACE plots and 370, 370, 363, and 370, in the Ambient/Control plots for 1992–3, 1993–4, 1995–6, and 1996–7, respectively.

Irrigation treatments

Using a strip-split-plot design, the four FACE and four Ambient plots were split into halves with each half receiving either an ample (Wet, 100% replacement of potential evapotranspiration) or a limiting (Dry, 50% of Wet) supply of irrigation water during the 1992–3 and 1993–4 experiments via a sub-surface drip irrigation system (Hunsaker *et al.*, 1996). The amounts of irrigation plus rain were 676 and 681 mm applied to the Wet plots for each of the two seasons, respectively (rain was 76 and 61 mm). The amounts of irrigation plus rain applied to the Dry plots were 351 and 318 mm. An ample amount of nitrogen fertilizer was applied to all plots (277 and 261 kg N ha⁻¹).

Nitrogen treatments

The same strip-split-plot design was used in 1995–6 and 1996–7, except N instead of water was the limiting factor (Kimball *et al.*, 1999). Amounts of N applied to the ample (High-N) plots were 350 kg N ha⁻¹ for both seasons, and to the limited (Low-N) plots were 70 and 15 kg N ha⁻¹ for 1995–6 and 1996–7, respectively. During 1996–7, an unfortunate mix-up of the fertilizer applications occurred in Replicate 3, and data from these plots have been excluded from all presentations in this paper. Ample irrigation amounts were applied in these FACE × N experiments.

Crop culture

Certified Yecora Rojo wheat seed was planted at mid-December in all seasons in east–west rows that were spaced 0.25 m apart (parallel to the drip irrigation tubing) (Kimball *et al.*, 1999). Fifty percent emergence of seedlings was observed about 1 January in all seasons, and FACE treatments commenced at that time. Air temperatures (2-m height) typically ranged from –5 to 42°C. Growing-degree-days amounted to about 2000, except for the Low-N treatments which matured earlier (Table 1 of Kimball *et al.*, 1999). Final harvests of grain occurred at the end of May for each season. The grain used in these quality tests came from the 18.1-m² ‘no-traffic’ subplot areas that were reserved for noninvasive measurements and the final harvests (Wall & Kimball, 1993). Representative

kilogram subsamples of grain from each plot each year were packaged and sent to the Western Wheat Quality Laboratory, USDA-ARS, Pullman, WA, USA for quality analyses.

Grain quality analyses

Upon receipt at the Western Wheat Quality Laboratory, all grain samples were frozen at –20°C for 2 d to kill any insects, then cleaned on a Carter dockage tester (Simon-Carter Co., Minneapolis, MN, USA), measured for test weight (Method 55–10; AACC, 2000), and scoured in a Forester and Son Cyclone Grain Scourer (Model 6, Forster and Son, Ada, OK, USA). Grain and flour moisture were determined according to Method 44–16 (AACC, 2000) (40 min for meal, 20 min for flour, both at 130°C). A subsample was ground in a UDY Cyclone (UDY Corp., Boulder, CO, USA) mill to pass a 0.5-mm screen. This ground meal was used for near infrared reflectance spectroscopy (NIR) grain hardness (Method 39–70 A; AACC, 2000) using a Technicon (Hoganas, Sweden) IA450 near infrared reflectance spectrometer, and grain protein (Dumas combustion method) (Method 46–30; AACC, 2000) using a Leco (St. Joseph, MI, USA) model FP-428. Grain protein is reported on a 12% moisture basis. Single kernel traits (hardness, moisture, weight and size) were obtained on a 300-kernel aliquot with a Perten (Perten Instruments North America, Springfield, IL, USA) model 4100 Single Kernel Characterization System.

Before milling, grain was tempered to 14.5% moisture content and held overnight. Milling was conducted on a modified Quadrumat (Brabender) system (Jeffers & Rubenthaler, 1979). Break flour (the amount of flour obtained early in the milling process from the break rollers. It is a measure of how easily the grain can be milled to flour, with higher values desired.) and straight-grade flour (the total amount of flour obtained) were expressed as per cent of total products. Flour ash was determined by Method 08–01 (AACC, 2000), and low values are desired. Milling score is a composite score that includes flour yield, break flour yield, and flour ash, with high values desired. Mixograph analysis was conducted on a 10-g sample at optimum water absorption (Method 54–40 A; AACC, 2000). (The mixing time requirement is a reliable index of loaf volume potential or protein quality, with medium to long mixing times associated with good loaf volumes (Finney *et al.*, 1987).) Ash and Mixograph absorption are expressed on a 14% flour moisture basis.

Bread baking used a straight-dough 100-g flour system at optimum water absorption and mixing time, 90-min fermentation and 75-ppm ascorbic acid (Method 10–10B; AACC, 2000). Determination of optimum water absorption (desirable to have high water absorption (Finney *et al.*, 1987)) and mixing time were judged by an experienced baker; internal crumb grain score was assigned on a scale of 1 (best) to 10 (unacceptable) by a three-member panel. Absorption is on a 14% flour moisture basis.

Protein-N yields

The total amounts of nitrogen in the grain protein harvested from the field (protein-N) were calculated from the protein concentrations times the grain yields in kg ha^{-1} , as reported by Pinter *et al.* (1997). A factor of 5.80 kg protein per kg N (Yamaguchi, 1992) was used to convert protein to nitrogen.

Statistical analysis

The data were analysed as a strip-split-plot design using the SAS 'Mixed' Procedure (Littell *et al.*, 1996) for the ANOVAs. The 1992–3 and 1993–4 FACE \times H₂O experiments were analysed separately from the 1995–6 and 1996–7 FACE \times N experiments. Year was handled as a repeated measure for both sets of experiments.

Results

Irrigation had relatively minor effects on grain quality (Table 1; 5th and 6th bars in panels of Figs 1, 2). For many of the tests the effects were small and not statistically significant (grain hardness (Fig. 1b), grain moisture (Fig. 1d), single kernel weight and size (Figs 1f,g), flour and break flour yields (Figs 1j,k), flour ash (Fig. 1l), milling score (Fig. 2a), mixograph absorption (Fig. 2b), bread crumb grain score (Fig. 2g)). Even when they were highly significant statistically, the changes due to the Dry treatment still were relatively small (test weight (–1%; Fig. 1a), single kernel hardness (–10% at Ambient and –2% at FACE; Fig. 1c), single kernel moisture (+1%; Fig. 1e), grain protein concentration (+2%; Fig. 1h), flour protein concentration (+4%; Fig. 1i), optimum mixing time for bread dough (+5%; Fig. 2e), and bread loaf volume (+3%; Fig. 2d), where all of these percentages are relative changes averaged over the CO₂ treatments, unless otherwise stated). Thus, the overall effect of drought in these experiments was a slight improvement in grain quality.

By contrast to irrigation, the Low-N nitrogen fertilizer treatment caused highly significant changes relative to High-N (Table 1, last two bars in the panels of Figs 1, 2), which were relatively large for many of the tests (test weight (+2%; Fig. 1a), grain hardness (–15%; Fig. 1b), single kernel hardness (–5%; Fig. 1c), grain protein (–36%; Fig. 1h), flour protein (–39%; Fig. 1i), flour yield (–2%; Fig. 1j), break flour yield (–9%; Fig. 1k), flour ash (+29%; Fig. 1l), milling score (–6%; Fig. 2a), mixograph and bake water absorption (–8%; Figs 2b,c), optimum mixing time for bread dough (+48%; Fig. 2e), bread loaf volume (–26%; Fig. 2d), and bread crumb grain score (+188%; Fig. 2g), where all of these percentages are relative changes averaged over the CO₂ treatments). Thus, Low-N, which had caused serious reductions in yield (Pinter *et al.*, 1997), also caused very serious reductions in both nutritional and baking quality of the wheat grain.

The effects of elevated CO₂ concentration (FACE; left 4 bars in the panels of Figs 1, 2) were often statistically significant but very much smaller than those due to Low-N (right 2 bars in Figs 1, 2), and there were several cases of significant interactions with both irrigation and nitrogen (Table 1, Figs 1, 2). First, at ample irrigation (Wet; 2nd bar in Figs 1, 2) and ample nitrogen (High-N; 4th bar in Figs 1, 2), there were changes in test weight (–1%; Fig. 1a), single kernel hardness (–9%, Wet only, Fig. 1c), grain and flour protein (–5% in Wet but 0% in High-N, Figs 2h,i), mixograph and bake water absorption (0%, only changed with Dry or Low-N, Figs 2b,c), optimum mixing time for bread dough (+6%, Fig. 2e), and bread loaf volume (–2% in Wet and 0% at High-N, Fig. 2d). The fact that the grain and flour protein concentrations and the bread loaf volume were unaffected by elevated CO₂ at High-N (with ample water) but that they decreased slightly under Wet (at ample N) suggests that perhaps our nitrogen application was not as ample as we intended in the FACE \times H₂O experiments. Recall that in the FACE \times H₂O experiments, the 'Ample' amount of N applied was about 270 kg ha^{–1}, whereas for the FACE \times N experiments, the High-N treatment received 350 kg N ha^{–1}. On the other hand, these results suggest that the slight deterioration in nutritional and baking quality caused by elevated CO₂ may be overcome by applying additional fertilizer.

We have shown that the Dry treatment tended to cause slight improvements in protein concentration and bread loaf volume (Figs 1h,i,d) while the FACE treatment tended to cause slight decreases. Consequently, the effects of FACE were smaller under the Dry treatment than they were under the Wet.

Conversely, under the Low-N treatment, with respect to Control, FACE exacerbated the deleterious effects of inadequate nitrogen on nutritional and baking quality. For example, grain protein decreased 33% at Ambient CO₂ and 39% under FACE (Fig. 1h), and loaf volume similarly decreased 22% at ambient and 29% under FACE (Fig. 2d).

Under Dry, the FACE treatment increased the yield of protein-N harvested by 18% relative to Ambient (1st bar, Fig. 2f), in spite of the 4% decrease in protein concentration in the grain (Fig. 1h). Under Wet, the protein-N yield increase due to FACE was 4% (2nd bar, Fig. 2f), in spite of the 5% decrease in protein concentration (Fig. 1h). There were equal yields of protein-N harvested from Control and FACE under Low-N (3rd bar, Fig. 2f), in spite of the 11% reduction in protein concentration under FACE (Fig. 1h). With High-N, there was no effect of FACE on protein concentration (Fig. 1h), so a 16% increase in grain yield with respect to Control (Pinter *et al.*, 1997) also resulted in a 16% increase protein-N yield (4th bar, Fig. 2f).

Discussion

The limited water (Dry), limited nitrogen (Low-N), and elevated CO₂ (FACE) treatments all affected the growth and grain yield of the wheat plants compared with those at ambient CO₂ (Ambient or Control) and ample water (Wet)

Table 1 Significance of elevated CO₂ (C), irrigation (I, i.e. water supply), nitrogen (N), Year (Y), and their interactions on various measures of wheat (*Triticum aestivum* L.) grain quality from the 1992–3 and 1993–4 FACE × H₂O experiments and from the 1995–6 and 1996–7 FACE × N experiments, as well as yields of protein-N harvested from the plots

Parameter	I or N	Nonstress Ambient or Control		Significance					
		Mean	SE	C	I or N	C*I or C*N	Y	C*Y	I*Y or N*Y
Test weight (kg m ⁻³)	I	819.0	1.4	**	***	***	***	*	ns
	N	811.4	2.6	ns	***	ns	***	ns	ns
Grain hardness (NIR value, dimensionless)	I	80.88	1.70	ns	ns	ns	*	ns	**
	N	95.27	3.07	ns	***	ns	***	ns	***
Single kernel hardness (dimensionless)	I	69.88	0.83	***	**	**	only in 1993–94		
	N	57.27	1.72	ns	*	ns	***	ns	***
Wheat grain moisture (% by weight)	I	9.56	0.01	ns	ns	ns	***	ns	ns
Single kernel moisture (% by weight)	I	11.30	0.04	ns	***	ns	only in 1993–94		
Single kernel weight (mg)	I	44.85	0.46	ns	ns	ns	only in 1993–94		
	N	39.05	1.37	ns	ns	ns	**	ns	ns
Single kernel size (mm)	I	3.09	0.02	ns	ns	ns	only in 1993–94		
	N	2.55	0.08	ns	ns	ns	**	**	ns
Protein concentration of grain (% by weight at 12% moisture)	I	14.98	0.09	***	**	**	***	ns	***
	N	14.95	0.16	***	***	***	ns	*	ns
Flour protein concentration (% by weight at 14% moisture)	I	13.15	0.07	***	**	ns	***	**	***
	N	13.61	0.13	***	***	***	ns	**	ns
Flour yield (% by weight of total products)	I	70.49	0.36	ns	ns	ns	***	ns	ns
	N	68.85	0.32	ns	***	ns	ns	ns	**
Break flour yield (% by weight of total products)	I	38.63	0.59	ns	ns	**	***	**	ns
	N	34.35	0.46	ns	***	*	***	**	***
Flour ash (% by weight at 14% moisture)	I	0.350	0.007	ns	ns	**	***	ns	**
	N	0.353	0.006	ns	***	**	ns	ns	ns
Milling score	I	87.74	0.46	ns	ns	*		ns	ns
	N	85.84	0.52	ns		*	ns	ns	
Mixograph absorption (% by weight corrected to 14% moisture)	I	64.18	0.37	**	ns	*	***	ns	*
	N	62.47	0.25	***	***	***	*	ns	ns
Bake water absorption (% by weight corrected to 14% moisture)	I	67.12	0.23	**	*	ns	***	ns	ns
	N	64.94	0.29	***	***	**	ns	ns	ns
Optimum mixing time for bread dough (min)	I	3.60	0.07	***	***	ns	***	ns	ns
	N	3.50	0.20	**	**	ns	***	*	*
Bread loaf volume (cm ³)	I	985	9	***	***	ns	ns	ns	***
	N	992	14	**	***	**	ns	ns	ns
Bread crumb grain score (1-excellent, 9-unsatisfactory)	I	3.12	0.14	ns	ns	ns	**	ns	ns
	N	2.55	0.42	ns	***	***	ns	ns	ns
Protein-N harvested from the field in the wheat grain (kg N ha ⁻¹)	I	232	6	**	***	**	***	ns	ns
	N	197	8	*	***	**	**	ns	*

I or N in the second column indicates which set of the experiments generated the data in that particular row. Also shown are the absolute means and SE for the nonstress Ambient or Control treatments (i.e. Ambient-Wet or Control-High-N). From these absolute values and the relative responses presented in Figs 1 and 2, the absolute values for the other treatments can be calculated. Probability levels are indicated by ns, *, **, and *** for 'not significant', 0.1, 0.05, and 0.01, respectively. Near infrared reflectance spectroscopy (NIR).

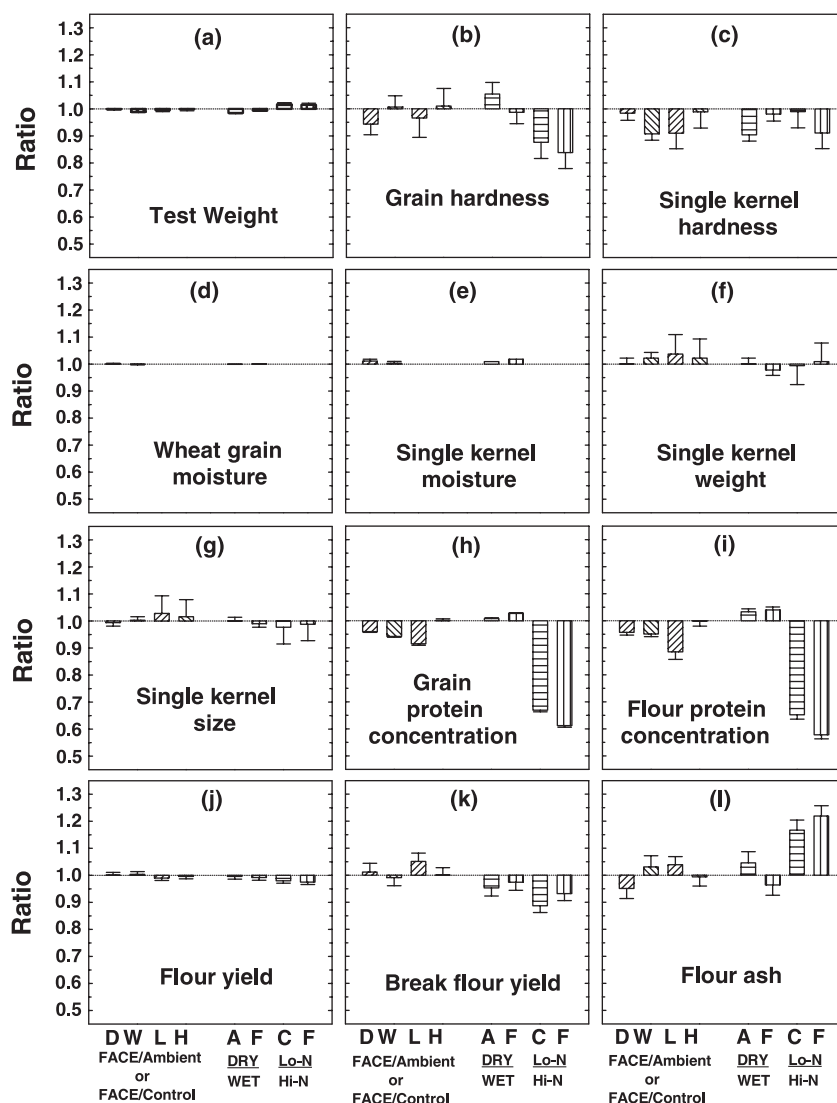


Fig. 1. Relative response ratios of wheat (*Triticum aestivum* L.) grain test weight (a), grain hardness (b), single kernel hardness (c), whole grain moisture (d), single kernel moisture (e), single kernel weight (f), single kernel size (g), grain protein concentration (h), flour protein concentration (i), flour yield (j), break flour yield (k), and flour ash (l) from free-air CO₂ enrichment (FACE) experiments. Wet and Dry irrigation levels were imposed in experiments conducted in 1992–3 and 1993–4, and Ambient daytime CO₂ levels were 370 $\mu\text{mol mol}^{-1}$, while CO₂ levels were controlled at 550 $\mu\text{mol mol}^{-1}$ in the FACE plots. Low and High nitrogen fertilizer levels were applied in experiments conducted in 1995–6 and 1996–7, and Control daytime CO₂ levels were again about 370 $\mu\text{mol mol}^{-1}$, while CO₂ levels were controlled at 200 $\mu\text{mol mol}^{-1}$ above ambient in the FACE plots. The left two bars in each panel are the FACE/Ambient response ratios under the Dry (D) and Wet (W) treatments, respectively. The 3rd and 4th bars are the FACE/Control ratios under the Low-N (L) and High-N (H) treatments, respectively. The 5th and 6th bars are the Dry/Wet response ratios under Ambient and FACE CO₂ treatments, respectively. The right two bars are the Low-N/High-N response ratios under Control and FACE CO₂ treatments, respectively. Bars indicate standard errors of the ratios, which were calculated from the standard errors of the individual means using the formula, $\Delta r = (ID\Delta N + IN\Delta D)D^{-2}$, where Δ indicates the standard error, D is the Ambient or Control mean value in the denominator, N is the FACE mean value in the numerator, and the vertical bars denote absolute values.

and nitrogen (High-N) (Pinter *et al.*, 1997). Dry and Low-N reduced yields by *c.* 33 and 21%, respectively. Under ample water and nitrogen, FACE increased yields about 16%. Under the Dry irrigation regime, FACE stimulated yields even more (*c.* 23%); whereas under Low-N, the stimulation due to FACE was smaller (*c.* 9%). Thus, with such effects occurring in the overall grain productivity of the wheat, some effects on grain quality might be expected.

Overall, the quality of grain produced under ample water and N and under drought was high (15.0% grain protein, Table 1). Smith & Gooding (1999), for example, state that 11.3% grain protein concentration (adjusted to 12% moisture) is the threshold commonly used by UK millers. Only the grain produced in our Low-N treatment was below this minimum acceptable value (10.00 and 9.15% \pm 0.16% under Control and FACE CO₂ treatments, respectively).

Although the FACE treatment caused decreases in grain protein concentration relative to Ambient or Control, except

for High-N (Fig. 1h), the increased grain yields due to FACE (Pinter *et al.*, 1997) resulted in greater harvests of protein-N when the N supply was high or ample. Thus, the overall production of protein-N was increased by elevated CO₂ except at Low-N (Fig. 2f, albeit at somewhat lower concentrations than produced in the Ambient or Control plots).

During the first two FACE \times H₂O experiments, we had Ambient plots with no blowers, while the FACE plots had blowers. Then during the second two FACE \times N experiments, we had Control and FACE plots all with blowers. However, in addition, during the 1995–6 growing season, two Ambient rings without blowers were established besides the four Control rings with blowers, as described by Pinter *et al.* (2000). Grain samples from these Ambient plots were subjected to the same battery of quality tests as those from the Control plots. The grain protein, bread loaf volume, and other parameters at both high and low levels of N were almost identical between the Ambient and Control plots (data not shown). Therefore,

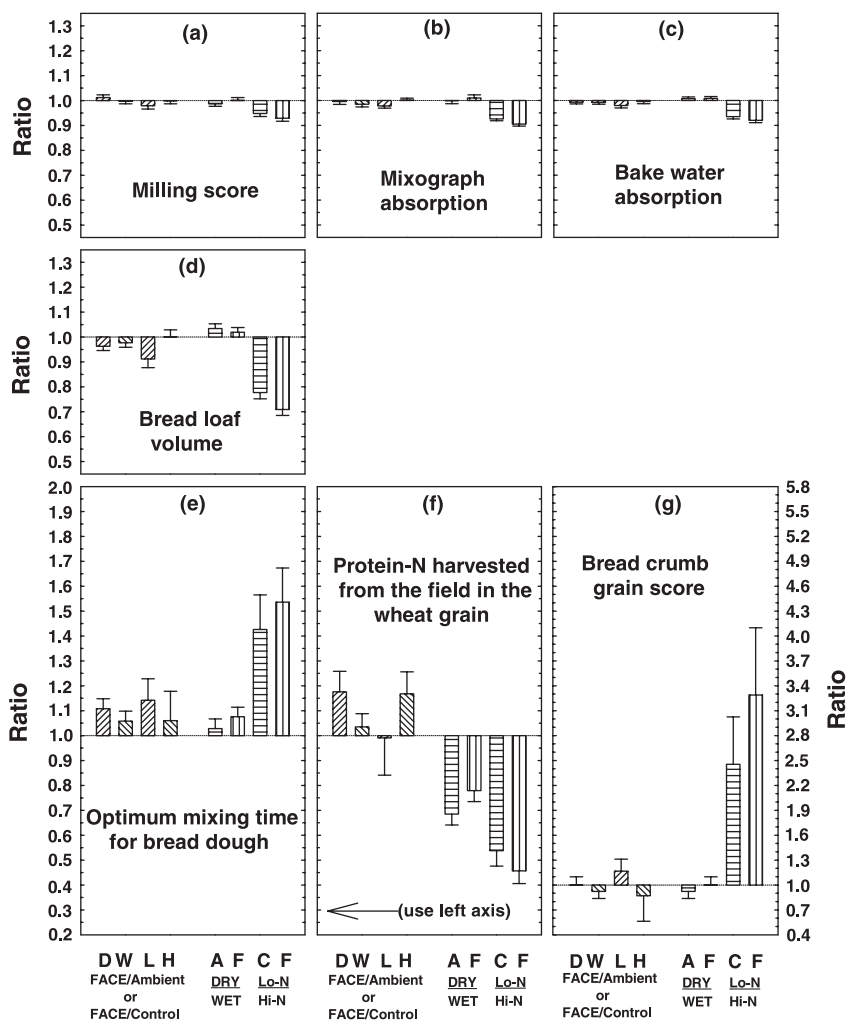


Fig. 2. Relative response ratios of wheat grain milling score (a), mixograph absorption (b), bake water absorption (c), bread loaf volume (d), optimum mixing time for bread dough (e), protein-N harvest from the field in the grain (f), and bread crumb grain score (g) from free-air CO₂ enrichment (FACE) experiments. The left two bars in each panel are the FACE/Ambient response ratios under the Dry (D) and Wet (W) treatments, respectively. The 3rd and 4th bars are the FACE/Control ratios under the Low-N (L) and High-N (H) treatments, respectively. The 5th and 6th bars are the Dry/Wet response ratios under Ambient and FACE CO₂ treatments, respectively. The right two bars are the Low-N/High-N response ratios under Control and FACE CO₂ treatments, respectively.

we conclude that the lack of blowers in the FACE \times H₂O experiments did not significantly affect grain quality.

The pattern of grain quality results is surprisingly similar to that of leaf N concentrations measured during the course of the same four FACE experiments by Sinclair *et al.* (2000). They found that drought had little effect on leaf N, as did elevated CO₂ under optimal conditions. However, the Low-N treatment caused a large decrease in leaf N compared with High-N, which was exacerbated by elevated CO₂. Thompson *et al.* (1997) measured the protein concentration of the grain from the 1995–6 experiment, and although not statistically significant, the trend was for FACE to decrease the concentration compared with Control. Their samples were smaller than those used herein. F. Porteous *et al.* (unpublished) similarly measured the N concentration of the whole shoots of the wheat plants sampled at the end of the 1996–7 experiment. Although CO₂ effects were not statistically significant, the trend again was for FACE to exacerbate the deleterious effects of the significant Low-N treatment. They did not detect any significant treatment effects on the N concentration of the

roots or of the grain, but their sample sizes also were much smaller than those used herein.

Our results are mostly in agreement with prior results reported in the literature, as reviewed in the Introduction of this paper. Like Barber & Jessop (1987) in Australia and Garrot *et al.* (1994) in Arizona, who both studied irrigated wheat, we found a slight improvement in grain quality with water stress. For about a 300-kg N ha⁻¹ reduction in applied N, we found about a 5% absolute decrease in protein concentration, or 1% per 60 kg N ha⁻¹, which is close to the 1% per 56 kg N ha⁻¹ determined by Benzie & Lane (1981) from many experiments in England. We mentioned that many of the prior experiments found little or no change in wheat grain nutritional or bread-making quality due to elevated levels of CO₂. On the other hand, a comparable number of such experiments did find decreases in the protein concentration, which reduced nutritional value and adversely affected baking quality. The data from these FACE experiments are basically consistent with these prior CO₂-enrichment data, suggesting that under conditions of optimum water and nitrogen, elevated

CO₂ will likely cause a small decrease in grain quality. However, quality is very sensitive to nitrogen supply, and if nitrogen were limiting even somewhat in the prior experiments (as may have been in our FACE × H₂O experiments), elevated CO₂ may cause a relatively larger decrease in grain quality.

It is difficult to assess how widely these results are applicable across the plant kingdom, across C₃ annual grasses, or even across varieties within the wheat species. Cotrufo *et al.* (1998) reviewed about 75 reports of the effects of elevated CO₂ on plant tissue N concentration. However, the research has mostly focused on leaves, and they list no data at all for grain or seeds or nuts, which is surprising considering how important seeds are to dispersal of plant species and to the diet of many animals. It is possible that some of the inconsistency among the wheat quality experiments mentioned previously has been due to differences in response among varieties. Benizian & Lane (1979, 1981, 1986) show substantial differences among wheat varieties with respect to absolute grain protein concentrations, but relative changes with respect to soil nitrogen supply were similar. Manderscheid *et al.* (1995) and Blumenthal *et al.* (1996) both included two varieties of wheat in their experiments. Manderscheid *et al.* (1995) found similar reductions in grain N concentration to elevated CO₂ between varieties, whereas Blumenthal *et al.* (1996) report a significant interaction with genotype. While these two studies found mean reductions in grain N concentration, we must remember several others found no significant effect of elevated CO₂ on grain quality. Considering the class of C₃ grasses, Manderscheid *et al.* (1995) also studied two varieties of barley, finding that one had reduced N content (but a smaller reduction than that of their wheat) while the other's did not change significantly. Similar variability exists among rice (*Oryza sativa* L.) experiments, with Ziska *et al.* (1997) reporting unspecified reductions of grain protein at elevated CO₂, while Seneweera *et al.* (1996) and Seneweera & Conroy (1997) found reductions of about 0.3% (absolute) in grain N concentration. Variability across other plant types may be similar. In a FACE experiment, which was very similar those reported herein, with cotton (*Gossypium hirsutum* L.), a C₃ woody perennial (cultivated as an annual for insect control), Prior *et al.* (1998) found absolute reductions in seed N concentration of c. 0.4% (7% relative). Thus, it appears that intra- and interspecific variations in effects of elevated CO₂ on grain quality exist. However, the results reported herein are certainly within the range of those in the literature, and they likely are representative of additional genotypes within wheat and other C₃ grasses and somewhat across the plant kingdom.

In conclusion, the data from these experiments suggest that adequate fertilizer is necessary to attain good quality grain and that, with ample fertilizer, the deleterious effects of elevated CO₂ will be minor. On the other hand, crops grown with limiting levels of N (such as is often the case in developing countries, or for other plants in unmanaged natural ecosystems) probably now have poorer quality grain than they could have, and future high CO₂ concentrations are likely to make the quality poorer yet.

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References

- AACC. 2000. *Approved methods of the american association of cereal chemists*, 10th edn. St. Paul, MN, USA: American Association of Cereal Chemists.
- Akey DH, Kimball BA. 1989. Growth and development of the beet armyworm on cotton grown in an enriched carbon dioxide atmosphere. *South-western Entomologist* 14: 255–260.
- Akey DH, Kimball BA, Mauney JR. 1988. Growth and development of the pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae), on bolls of cotton grown in enriched carbon dioxide atmospheres. *Environmental Entomology* 17: 452–455.
- Barber JS, Jessop RS. 1987. Factors affecting yield and quality in irrigated wheat. *Journal of Agriculture Science, Camb* 109: 19–26.
- Benizian B, Lane P. 1979. Some relationships between grain yield and grain protein of wheat experiments in South-east England and comparisons with such relationships elsewhere. *Journal of Science Food Agriculture* 30: 59–70.
- Benizian B, Lane P. 1981. Interrelationship between nitrogen concentration in grain, grain yield and added fertilizer nitrogen in wheat experiments of South-east England. *Journal of Science Food Agriculture* 32: 35–43.
- Benizian B, Lane P. 1986. Protein concentration of grain in relation to some weather and soil factors during 17 years of English winter-wheat experiments. *Journal of Science Food Agriculture* 37: 435–444.
- Blumenthal C, Rawson HM, McKenzie E, Gras PW, Barlow EWR, Wrigley CW. 1996. Changes in wheat grain quality due to doubling the level of atmospheric CO₂. *Cereal Chemistry* 73: 762–766.
- Conroy JP, Seneweera S, Basra AS, Rogers G, Nissen-Wooler B. 1994. Influence of rising atmospheric CO₂ concentrations and temperature on growth, yield, and grain quality of cereal crops. *Australian Journal of Plant Physiology* 21: 741–758.
- Cotrufo MF, Ineson P, Scott A. 1998. Elevated CO₂ reduces the nitrogen concentration of plant tissues. *Global Change Biology* 4: 43–54.
- Dijkstra P, Schapendonk AHCM, Groenwold KO, Jansen M, van de Geijn SC. 1999. Seasonal changes in the response of winter wheat to elevated atmospheric CO₂ concentration grown in open-top chambers and field tracking chambers. *Global Change Biology* 5: 563–576.
- Finney KF, Yamazaki WT, Youngs VL, Rubenthaler GL. 1987. Quality of hard, soft, and durum wheats. In: Heyne EG, ed. *Wheat and wheat*

- improvement. Madison, WI, USA: American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, 677–748.
- Garrot DJ, Ottman MJ, Fangmeier DD, Husman SH. 1994. Quantifying wheat water stress with crop water stress index to schedule irrigations. *Agronomy Journal* 86: 195–199.
- Hakala K. 1998. Growth and yield potential of spring wheat in a simulated changed climate with increased CO₂ and higher temperature. *European Journal of Agronomy* 9: 41–52.
- Havelka UD, Wittenbach VA, Boyle MG. 1984. CO₂-enrichment effects on wheat yield and physiology. *Crop Science* 24: 1163–1168.
- Hendrey GR, ed. 1993. *Free-air carbon dioxide enrichment for plant research in the field*. Boca Raton, FL, USA: C. K. Smoley.
- Hendrix DL. 1992. Influence of elevated CO₂ of leaf starch of field-grown cotton. *Critical Reviews in Plant Science* 11: 223–226.
- Hesman T. 2000. Greenhouse gassed: carbon dioxide spells indigestion for food chains. *Science News* 157: 200–202.
- Hunsaker DJ, Kimball BA, Pinter Jr PJ, LaMorte RL, Wall GW. 1996. CO₂ enrichment and irrigation effects on wheat evapotranspiration in a free-air environment. *Transactions of the Of the ASAE* 39: 1345–1355.
- Intergovernmental Panel on Climate Change. 1996. *Climate change 1995: summary for policy makers and technical summary of the working group I report*. Houghton Cambridge, UK: Cambridge University Press.
- Jeffers HC, Rubenthaler GL. 1979. Effects of roll temperature on flour yield with the Brabender Quadrumat Experimental mills. *Cereal Chemistry* 54: 1018–1025.
- Kimball BA. 1983. Carbon dioxide and agricultural yield: an assemblage and analysis of 430 prior observations. *Agronomy Journal* 75: 779–788.
- Kimball BA. 1986. Influence of elevated CO₂ on crop yield. In: Enoch HZ, Kimball BA, eds. *Carbon dioxide enrichment of greenhouse crops, vol 2: physiology, yield, and economics*. Boca Raton, FL, USA: CRC Press, Inc., 105–115.
- Kimball BA, LaMorte RL, Pinter Jr PJ, Wall GW, Hunsaker DJ, Adamsen FJ, Leavitt SW, Thompson TL, Matthias AD, Brooks TJ. 1999. Free-air CO₂ enrichment (FACE) and soil nitrogen effects on energy balance and evapotranspiration of wheat. *Water Resources Research* 35: 1179–1190.
- Kimball BA, Pinter Jr PJ, Garcia RL, LaMorte RL, Wall GW, Hunsaker DJ, Wechsung G, Wechsung F, Kartschall Th. 1995. Productivity and water use of wheat under free-air CO₂ enrichment. *Global Change Biology* 1: 429–442.
- Kimball BA, Pinter Jr PJ, Wall GW, Garcia RL, LaMorte RL, Jak PMC, Frumau KFA, Vugts HF. 1997. Comparisons of responses of vegetation to elevated carbon dioxide in free-air and open-top chamber facilities. In: Allen Jr LH, Kirkham MB, Olszyk DM, Whitman CE, eds. *Advances in carbon dioxide research*. Madison, WI, USA: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, 113–130.
- Lawlor DW, Mitchell RAC. 2000. Crop ecosystem responses to climatic change: Wheat. In: Reddy KR, Hodges HF, eds. *Climate change and global crop productivity*. New York, USA: CABI Publishing, 57–80.
- Lincoln DE. 1993. The influence of plant carbon dioxide and nutrient supply on susceptibility to insect herbivores. In: Rozema J, Lambers H, van de Geijn SC, Cambridge ML, eds. *CO₂ and biosphere*. Dordrecht, The Netherlands: Kluwer Academic Publishers, 273–280.
- Littell RC, Milliken GA, Stroup WW, Wolfinger RD. 1996. *SAS system for mixed models*. Cary, NC, USA: SAS Institute, Inc.
- Manderscheid R, Bender JJ, Jäger H-J, Weigel HJ. 1995. Effects of season long CO₂ enrichment on cereals. II. Nutrient concentrations and grain quality. *Agriculture, Ecosystems and Environment* 54: 175–185.
- Monje O, Bugbee B. 1998. Adaptation to high CO₂ concentration in an optimal environment: radiation capture, canopy quantum yield and carbon use efficiency. *Plant, Cell & Environment* 21: 315–324.
- Pinter Jr PJ, Kimball BA, Garcia RL, Wall GW, Hunsaker DJ, LaMorte RL. 1996. Free-air CO₂ enrichment: responses of cotton and wheat crops. In: Koch GW, Mooney HA, eds. *Carbon dioxide and terrestrial ecosystems*. San Diego, CA, USA: Academic Press, 215–248.
- Pinter Jr PJ, Kimball BA, Wall GW, LaMorte RL, Adamsen F, Hunsaker DJ. 1997. *Effects of elevated CO₂ and soil nitrogen fertilizer on final grain yields of spring wheat*. Annual Research Report. Phoenix, USA: U.S. Water Conservation Laboratory, USDA, Agricultural Research Service, 71–74.
- Pinter Jr PJ, Kimball BA, Wall GW, LaMorte RL, Hunsaker DJ, Adamsen FJ, Frumau KFA, Vugts HF, Hendrey GR, Lewin KF, Nagy J, Johnson JB, Wechsung F, Leavitt SW, Thompson TL, Matthias AD, Brooks TJ. 2000. Free-air CO₂ enrichment (FACE): blower effects on wheat canopy microclimate and plant development. *Agricultural and Forest Meteorology* 103/4: 319–332.
- Prior SA, Torbert HA, Runion GB, Mullins GL, Rogers HH, Mauney JR. 1998. Effects of carbon dioxide enrichment on cotton nutrient dynamics. *Journal of Plant Nutrition* 21: 1407–1426.
- Reuveni J, Bugbee B. 1997. Very high CO₂ reduces photosynthesis, dark respiration and yield in wheat. *Annals of Botany* 80: 539–546.
- Rogers GS, Gras PW, Batey IL, Milham PJ, Payne L, Conroy JP. 1998. The influence of atmospheric CO₂ concentration on the protein, starch, and mixing properties of wheat flour. *Australian Journal of Plant Physiology* 25: 387–393.
- Seneweera S, Blakeney A, Milham P, Basra AS, Barlow EWR, Conroy J. 1996. Influence of rising atmospheric CO₂ and phosphorous nutrition on the grain yield and quality of rice (*Oryza sativa* cv. Jarrah). *Cereal Chemistry* 73: 239–243.
- Seneweera SP, Conroy JP. 1997. Growth, grain yield and quality of rice (*Oryza sativa* L.) in response to elevated CO₂ and phosphorus. *Soil Science Plant Nutrition* 43: 1131–1136.
- Sinclair TR, Pinter Jr PJ, Kimball BA, Adamsen FJ, LaMorte RL, Wall GW, Hunsaker DJ, Adam N, Brooks TJ, Garcia RL, Thompson T, Leavitt S, Matthias A. 2000. Leaf nitrogen concentration of wheat subjected to elevated [CO₂] and either water or N deficits. *Agriculture, Ecosystems and Environment* 79: 53–60.
- Smith GP, Gooding MJ. 1999. Models of wheat grain quality considering climate, cultivar and nitrogen effects. *Agricultural and Forest Meteorology* 94: 159–170.
- Stone PJ, Savin R. 1999. Grain quality and its physiological determinants. In: Satorre EH, Slafer GA, eds. *Wheat: ecology and yield determination*. New York, USA: Food Products Press, 85–120.
- Tester RF, Morrison WR, Ellis RH, Piggett JR, Batts GR, Wheeler TR, Morison JIL, Hadley P, Ledward DA. 1995. Effects of elevated growth temperature and carbon dioxide levels on some physiochemical properties of wheat starch. *Journal of Cereal Science* 22: 63–71.
- Thompson TL, White SA, Leavitt SW, Pendall E, Matthias AD. 1997. Nitrogen and carbon dynamics in a wheat agroecosystem under elevated atmospheric CO₂. In: Mortvedt JJ, ed. *Proceedings Dahlia Greidinger International Symposium on Fertilizer and Environment*. 24–27 March, 1997. Haifa, Israel: Technion-Israel Institute of Technology, 182–192.
- Thompson GB, Woodward FI. 1994. Some influences of CO₂ enrichment, nitrogen nutrition and competition on grain yield and quality in spring wheat and barley. *Journal of Experimental Botany* 45: 937–942.
- Wall GW, Kimball BA. 1993. Biological databases derived from free air carbon dioxide enrichment experiments. In: Schulze ED, Mooney HA, eds. *Design and execution of experiments on CO₂ enrichment*. Brussels, Belgium: Commission of the European Communities, 329–351.
- Williams M, Shewry PR, Lawlor DW, Harwood JL. 1995. The effects of elevated temperature and atmospheric carbon dioxide concentration on the quality of grain lipids in wheat (*Triticum aestivum* L.) grown at two levels of nitrogen application. *Plant, Cell & Environment* 18: 999–1009.
- Yamaguchi M. 1992. Determination of the nitrogen-to-protein conversion factor in cereals. In: Linskens H-F, Jackson JF, eds. *Seed analysis*. Berlin, Germany: Springer Verlag, 95–107.
- Ziska LH, Namuco O, Moya T, Quilang J. 1997. Growth and yield response of field-grown tropical rice to increasing carbon dioxide and temperature. *Agronomy Journal* 89: 45–53.